

**Amendments to the Claims:**

Please amend claims 1, 2, 6, 11, 12, 23, 31, 38, 50 and 60 as indicated below.

Please cancel claims 7, 8, 32, 33, 40, 80-82 and 87 without prejudice.

The listing of claims will replace all prior version, and listings, of claims in the application:

**Listing of Claims:**

1. (Currently Amended) [[A]] An *in vitro* method of detecting a protease activity in a cell, comprising:

a) providing a cell comprising:

- i) at least one destabilization domain, wherein said destabilization domain is non-cleavable by [[I]] $\alpha$ -NH-ubiquitin protein endoproteases;
- ii) a reporter moiety; and
- iii) a linker moiety that operatively couples said destabilization domain to said reporter moiety,

wherein said linker moiety comprises a protease cleavage site for said protease activity and cleavage of said linker moiety by said protease activity decreases the coupling of said destabilization domain to said reporter moiety thereby increasing the stability of said reporter moiety, and

wherein said linker moiety is non-cleavable by said [[I]] $\alpha$ -NH-ubiquitin protein endoproteases, and wherein the destabilization domain, the reporter moiety, and the linker are encoded by one or more nucleic acid molecules in the cell; and

b) detecting said reporter moiety, or a product of said reporter moiety, thereby detecting the protease activity in the cell.

2. (Currently Amended) The method of claim 1, wherein said at least one destabilization domain is arranged as a linear multimer, and wherein said linear multimer comprises at least two copies of said destabilization domain and is non-cleavable by said  $[[I]]\alpha$ -NH-ubiquitin protein endoproteases.
3. (Previously Presented) The method of claim 1, wherein said linker moiety is a non-naturally occurring polypeptide or protein.
4. (Previously Presented) The method of claim 1, wherein said linker moiety covalently couples said destabilization domain to said reporter moiety.
5. (Original) The method of claim 1, wherein said linker moiety is between about 1 and 30 amino acid residues.
6. (Currently Amended) The method of claim 1, wherein said destabilization domain comprises a ubiquitin homolog, ~~wherein the amino acid sequence of the homolog is at least 85% identical to~~ comprising a mutation at glycine 76 of the amino acid sequence of wild-type ubiquitin.
7. (Canceled)
8. (Canceled)
9. (Original) The method of claim 1, wherein said linker moiety comprises a first amino acid sequence that is covalently coupled to said reporter moiety, and a second amino acid sequence that is covalently coupled to said at least one destabilization domain.
10. (Canceled)
11. (Currently Amended) The method of claim 1, wherein said reporter moiety is selected from the group consisting of a naturally fluorescent protein ~~or homolog thereof~~,  $[[9]]\beta$ -

lactamase, [[9]] $\beta$ -galactosidase, alkaline phosphatase, chloramphenicol  
acetyltransferase,  $\beta$ -glucuronidase, peroxidase, and luciferase.

12. (Currently Amended) The method of claim 11, wherein said reporter moiety comprises a [[9]] $\beta$ -lactamase.
13. (Previously Presented) The method of claim 11, wherein said reporter moiety comprises an *Aequorea* Green fluorescent protein.
14. (Previously Presented) The method of claim 11, wherein said reporter moiety comprises an Anthozoan Green fluorescent protein.
15. (Original) The method of claim 1, wherein said cell is a mammalian cell.
16. (Original) The method of claim 1, wherein said cell is a yeast cell.
17. (Original) The method of claim 1, wherein said cell is an insect cell.
18. (Original) The method of claim 1, wherein said cell is a plant cell.
19. (Original) The method of claim 1, wherein said method further comprises the step of adding a protein synthesis inhibitor to said cell.
20. (Original) The method of claim 1, wherein said method further comprises the step of adding an inhibitor of said reporter moiety to said cell.
21. (Original) The method of claim 1, wherein said method further comprises the step of adding a test chemical to said cell.
22. (Previously Presented) The method of claim 21, wherein said method further comprises the step of relating said reporter moiety activity before addition of said test chemical to said reporter moiety activity after addition of said test chemical.

23. (Currently Amended) [[A]] An *in vitro* method of increasing the concentration of one or more target proteins in a cell, comprising:
- a) providing a cell comprising,
    - i) a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by [[I]] $\alpha$ -NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain;
    - ii) a target protein; and
    - iii) a linker that operatively couples said linear multimerized destabilization domain to said target protein,
      - wherein said linker comprises a protease cleavage site for a protease and cleavage of said linker by said protease decreases the coupling of said linear multimerized destabilization domain to said target protein, and wherein the destabilization domain, the target protein, and the linker are encoded by one or more nucleic acid molecules in the cell, thereby increasing the stability of said target protein in said cell, and
      - wherein said linker is non-cleavable by [[I]] $\alpha$ -NH-ubiquitin protein endoproteases; and
  - b) providing said protease to cause cleavage of said linker thereby increasing the stability and concentration of said target protein in said cell.
24. (Original) The method of claim 23, wherein said protease is naturally expressed in said cell.
25. (Original) The method of claim 23, wherein said protease is not naturally expressed in said cell.
26. (Original) The method of claim 23, further comprising the step of adding an inhibitor of said protease.

27. (Original) The method of claim 23, wherein said linker is between 1 and 30 amino acid residues.
28. (Original) The method of claim 23, wherein said cell is a mammalian cell.
29. (Original) The method of claim 23, wherein said cell is a yeast cell.
30. (Original) The method of claim 23, wherein said cell is an insect cell.
31. (Currently Amended) The method of claim 23, wherein said destabilization domain comprises a ubiquitin homolog, ~~wherein the amino acid sequence of the homolog is at least 85% identical to~~ comprising a mutation of glycine 76 of the amino acid sequence of wild-type ubiquitin.
32. (Canceled)
33. (Canceled)
34. (Original) The method of claim 23, wherein said protease is provided by transfecting said cell with an expression vector comprising a nucleic acid sequence encoding said protease.
35. (Original) The method of claim 34, wherein said expression vector further comprises an inducible promoter.
36. (Original) The method of claim 34, wherein said expression vector is a retroviral expression vector.
37. (Original) The method of claim 34, wherein said protease is a viral protease.
38. (Currently Amended) [[A]] An in vitro method of destabilizing a target protein in a cell, comprising:
  - a) introducing into the cell, one or more nucleic acid molecules that together encode a target protein and a linear multimerized destabilization domain, wherein the target protein is

operatively coupled to a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by [[I]] $\alpha$ -NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain, and wherein said destabilization domain comprises a ubiquitin homolog having a mutation at glycine 76 of an amino acid sequence at least 85% identical to the amino acid sequence of wild-type ubiquitin; and

b) allowing the target protein to be recognized by one or more elements of a cellular protein degradation apparatus, thereby destabilizing the target protein.

Claims 39-49 (Canceled)

50. (Currently Amended) A recombinant DNA molecule, comprising a nucleic acid sequence encoding:

- a) a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by [[I]] $\alpha$ -NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain, wherein at least one copy of the destabilization domain comprises a mutation of glycine 76 of the amino acid sequence of wild-type ubiquitin;
- b) a target protein; and
- c) a linker moiety that operatively couples said multimerized destabilization domain to said target protein,  
wherein said linker is non-cleavable by [[I]] $\alpha$ -NH-ubiquitin protein endoproteases.

Claims 51-54 (Canceled)

55. (Withdrawn) A recombinant protein molecule, comprising an amino acid sequence encoding for;

- a) a linear multimerized destabilization domain, wherein said multimerized destabilization domain is non-cleavable by I-NH-ubiquitin protein

endoproteases, and comprises at least two copies of said destabilization domain,

- b) a target protein, and
- c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a I-NH-ubiquitin protein endoproteases.

Claims 56-59 (Canceled)

60. (Currently Amended) [[A]] An isolated host cell, comprising a nucleic acid sequence encoding;

- a) a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by [[I]] $\alpha$ -NH-ubiquitin protein endoproteases, and comprises at least two copies of said destabilization domain, wherein at least one copy of the destabilization domain comprises a mutation of glycine 76 of the amino acid sequence of wild-type ubiquitin;
- b) a target protein; and
- c) a linker moiety that operatively couples said linear multimerized destabilization domain to said target protein,  
wherein said linker is non-cleavable by  $\alpha$ -NH-ubiquitin protein endoproteases, and wherein said linker moiety comprises a protease recognition site.

Claims 61-82 (Canceled)

83. (Previously Presented) The method of claim 1, wherein said cell is from an organism other than a transgenic organism.

84. (Previously Presented) The method of claim 38, wherein said cell is from an organism other than a transgenic organism.

85. (Previously Presented) The method of claim 1, wherein said cell is from a transgenic rodent.

86. (Previously Presented) The method of claim 1, wherein said cell is from a transgenic plant.

87. (Canceled)